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Crystallization of the Fab Fragment of a Monoclonal Anti-Spin-Label Antibody With and Without Bound Hapten

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ABSTRACT

The F_{ab} fragment of a monoclonal antibody, AN02, specific for a spin-labelled dinitrophenyl hapten was crystallized both with and without bound hapten. Both crystals formed in phosphate buffered saline (150mM NaCl, 10mM Na_2PO_4, .02% NaN_3, pH 7.4) at 4°C and diffract to at least 2.2 Å resolution. F_{ab} with bound hapten crystallizes in space group $P6_122$ or $P6_522$ with cell dimensions a=b=73 Å and c=373 Å. Unoccupied F_{ab} also crystallizes in space group $P6_122$ or $P6_522$ but with cell dimensions a=b=75 Å and c=376 Å.

– The structural change that an antibody undergoes upon binding of antigen is interesting in terms of antibody diversity and function. Independent crystal structures of an antibody both with and without antigen have not yet been solved. We report here the crystallization of the F_{ab} fragment of AN02, a monoclonal antibody specific for a spin-labelled dinitrophenyl hapten, with and without hapten (for the chemical structure of this hapten see Anglister et al. (1984a)).

In addition to allowing assessment of the effect of hapten binding on antibody structure, solution of the AN02 crystal structures will enable a comparison of x-ray and NMR structural information. The bound spin-label hapten broadens NMR signals of nearby (<17Å) protons in a strongly distance dependent manner. An NMR difference spectrum, F_{ab} alone minus F_{ab} with bound spin-label, is dominated by resonances from protons near the electron spin. Culturing the antibody producing cells in media containing specific deuterated amino acids results in virtually complete incorporation of the deuterated amino acids into the antibody. The spin-label's broadening effect, in combination with deuteration, has enabled identification of NMR signals from amino acids in the hapten binding site and measurement of the distance between many of these residues and the electron spin (Anglister et al., 1984a;1984b;1987).

The genes coding for the AN02 heavy and light chains have recently been cloned (Leahy et al., 1988), and a panel of AN02 mutants is currently being constructed using site directed mutagenesis. The ability to use NMR, x-ray, and recombinant DNA techniques makes AN02 and its mutants a rich source for study of structural and functional aspects of the antibody combining site.

The F_{ab} fragment was prepared as described previously for NMR studies (Anglister et al., 1984a). Briefly, whole antibody was isolated from tissue culture supernatants with a protein A-sepharose column. The antibody was concentrated by vacuum dialysis and digested with papain. The F_{ab} fragment was separated from the F_c fragment and smaller digestion products by chromatography on a G-75 sephadex column followed by a protein A-sepharose column. The product was concentrated to 20 mg/ml and dialysed into phosphate buffered saline (150mM NaCl, 10mM Na_2PO_4, .02% NaN_3, pH 7.4). To prepare samples of

F_{ab} with hapten, an excess of solid hapten was added to the F_{ab} and the mixture gently rotated at room temperature for four hours. The sample was then spun in a microfuge at 15,000 rpm for 20 min and the hapten saturated F_{ab} supernatant removed. From UV absorption spectra we estimate that less than .01% of the F_{ab} in these samples is free of hapten. The F_{ab} samples were centrifuged a second time for 20 min at 15,000 rpm in a microfuge, and the solution aliquotted into 5 mm glass NMR tubes. The samples were then maintained at 4°C.

Crystals formed after approximately two weeks with different tubes containing different sized crystals. Most crystals grew as hexagonal barrels with the longest dimension along the six-fold axis. Either many small crystals coated the NMR tubes and did not appear to grow significantly after first observation, or a few crystals appeared which then continued to grow to a much larger size. In cases where a few large crystals appeared, crystals grew to an average size of 0.5x0.5x0.7mm. One crystal grew to approximately 2x2x3mm.

Crystals were mounted in quartz capillaries and screened precession photographs (μ=15°) of the hk0, h0l, and 111 planes were taken using rotating anode radiation. Crystals of AN02 F_{ab} both with and without hapten crystallized in space group $P6_122$ or $P6_522$. The cell dimensions for F_{ab} alone are a=b=75 Å and c=376Å, and the cell dimensions for F_{ab} with the spin-label hapten bound are a=b=73Å and c=373 Å. Both crystals withstand x-rays well and diffract to greater than 2.2 Å resolution, making them suitable for x-ray crystallographic studies.

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